

Isolation of Lung Immune components

Lecture 8

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Isolation of mononuclear cells from lung

In order to isolate the lung immune cells we should provide the followings

- 1- Complete media (RPMI +Fetal Bovine serum 2%)
- 2- Phosphate Buffer Saline (PBS)
- 3- RBC lysis
- 4-Histopaque
- 5- Centrifuge
- 6- Stomacher (smasher)
- 7-Vortex
- 8- Pipette
- 9- Filters (different sizes)
- 10-Tubes (50 ml size)
- 11-Trypan blue stain
- 12- Cell Counter

Procedure for Isolation of Lung Immune cells

- 1- Put 2 lungs of each couple mouse into bags contain 10 ml of complete media (Because they are few in there number when we isolated them)
- 2- Smash the lung by stomacher for 10 minutes
- 3- Turn the content into 10 ml tubes then filter the content
- 4- Centrifugate the tubes at 1300 rpm/for 7 minutes at 4C.
- 5- Discard the supernatant
- 5- Add 600 microliter of RBC lysis for 45 seconds only and vortex then add 10 ml of media ovel all the content then vortex the tube. Again.
- 6- Centrifugate it for 1000 rpm/for 10 minutes at 4C .

Procedure for Isolation of Lung Immune cells

7-Wash the cells by Adding 5 ml of Phosphate Buffer Saline PBS

8- Discard the supernatant then add 5 ml of PBS .

9-Layering stage : On another new 10 ml tube, put 5 ml of Hisopaque and leave it for room temprature then pipette the 5 ml of lung cells and added gently and very slowly over the 5 ml histopaque then centrifugate it on 500 xg for 30 minutes and temprature of 25 C

10- Collect the middle layer of the tube where the immune cells in this layer then add 10 ml PBS then centrifugate it on 1300 rpm for 7 minutes for washing the cells

11- Discard the supernatant

12- Add 5 ml of PBS

13- Check the viability and count the cells by cells counter

14- Start then whatever you want of any immune assay.

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Best
Wishes!

The image features the phrase "Best Wishes!" written in a bold, black, cursive script. The text is set against a white, rectangular background that is tilted at an angle. The words "Best" and "Wishes!" are stacked vertically. The lettering is thick and expressive, with a prominent shadow effect that gives it a three-dimensional appearance. Scattered around the text are several small, five-pointed gold stars. A long, sweeping, curved flourish extends from the bottom of the word "Wishes!" across the width of the text. The entire composition is centered on a light blue gradient background.